

RESPONSE TO RESTRICTION AND ELECTION OF SPECIES REQUIREMENTS AND
PRELIMINARY AMENDMENT

Attorney Docket No.: Q96125

Application No.: 10/587,398

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (Original) A method for isolating and culturing multipotent progenitor/stem cells from cord blood-derived mononuclear cells, which comprises culturing the cord blood-derived mononuclear cells successively in:
 - 1) a first animal cell culture medium comprising fetal bovine serum(FBS), L-glutamine and granulocyte macrophage-colony stimulating factor(GM-CSF), in addition to inorganic salts, vitamins, amino acids and/or supplementary elements;
 - 2) a second animal cell culture medium which is the same as the first animal cell culture medium except for lacking GM-CSF; and
 - 3) a third animal cell culture medium which is the same as the first animal cell culture medium except that GM-CSF is replaced with stem cell factor(SCF) and endothelial growth factor(EGF).
2. (Original) The method of claim 1, wherein the animal cell culture medium further contains D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

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3. (Original) The method of claim 1, wherein the first animal cell culture medium contains 10 to 20% FBS, 1 to 2 mM L-glutamine, 10 to 100 ng/M ℓ GM-CSF; the second animal cell culture medium contains 10 to 20% FBS and 1 to 2 mM L-glutamine; and the third animal cell culture medium contains 10 to 20% FBS, 1 to 2 mM L-glutamine, 10 to 100 mg/M ℓ SCF and 10 to 50 mg/M ℓ EGF.

4. (Original) The method of claim 1, wherein the cultivation in the first animal cell culture medium is conducted by inoculating the mononuclear cells into the first animal cell culture medium at a concentration of 1×10^5 to 1×10^6 cells/cm 2 and culturing at 37°C under an atmosphere of 5% CO₂ for 1 to 2 weeks; the cultivation in the second animal cell culture medium is conducted by replacing the first animal cell culture medium by the second animal cell culture medium after confirming the formation of a multi-layer cell colony and further culturing at 37°C under an atmosphere of 5% CO₂ for 1 to 2 weeks; and the cultivation in the third animal cell culture medium is conducted by inoculating the cells cultured in the second animal cell culture medium into the third animal cell culture medium at a concentration of 2×10^4 to 8×10^4 cells/cm 2 after observing the metamorphosis of the multi-layer cell colony into a mono-layer cell colony and further culturing at 37°C under an atmosphere of 5% CO₂ for 1 to 2 weeks.

5. (Currently amended) A multipotent progenitor/stem cell isolated and cultured from a cord blood-derived mononuclear cell, according to the method of claim 1, the multipotent progenitor/stem cell is isolated and cultured by a method comprising

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culturing the cord blood-derived mononuclear cells successively in:

1) a first animal cell culture medium comprising fetal bovine serum(FBS), L-glutamine and granulocyte macrophage-colony stimulating factor(GM-CSF), in addition to inorganic salts, vitamins, amino acids and/or supplementary elements;

2) a second animal cell culture medium which is the same as the first animal cell culture medium except for lacking GM-CSF; and

3) a third animal cell culture medium which is the same as the first animal cell culture medium except that GM-CSF is replaced with stem cell factor(SCF) and endothelial growth factor(EGF),

wherein said multipotent progenitor/stem cell has an immunophenotype profile showing positive reactions against antibodies for CD14, CD31, CD44 and CD45 antigens; negative reactions against antibodies for CD34, CD62E, CD90(Thy-1) and CD133 antigens; positive and partial positive reactions against antibodies for CD54 and CD166 antigens; negative and partial negative reactions against antibodies CD73(SH3, SH4) and CD105(SH2) antigens; and negative and partial positive reactions against antibodies for CD49a and CD104 antigens.

6. (Canceled)

7. (Original) An animal cell culture medium composition for isolating and culturing multipotent progenitor/stem cells from cord blood-derived mononuclear cells, which is selected from the group consisting of:

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an animal cell culture medium composition comprising 10 to 20% FBS, 1 to 2 mM L-glutamine and 10 to 100 ng/Mℓ GM-CSF;

an animal cell culture medium composition comprising 10 to 20% FBS and 1 to 2 mM L-glutamine; and

an animal cell culture medium composition comprising 10 to 20% FBS, 1 to 2 mM L-glutamine, 10 to 100 mg/Mℓ SCF and 10 to 50 mg/Mℓ EGF,

wherein each of the animal cell culture media further contains inorganic salts, amino acids, vitamins and/or supplementary factors.

8. (Original) The animal cell culture medium composition of claim 7, wherein the animal cell culture medium further contains D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

9. (Original) A method for differentiating multipotent progenitor/stem cells of claim 5 into neurons, which comprises culturing the multipotent progenitor/stem cells in an animal cell culture medium comprising FBS, L-glutamine, retinoic acid, folskolin, nerve growth factor(NGF), a supplementary element mixture and beta-mercaptoethanol, in addition to D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

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10. (Original) The method of claim 9, wherein the animal cell culture medium contains 0.1 to 2% FBS, 1 to 2 mM L-glutamine, 1 to 25 μ M retinoic acid, 1 to 20 μ M forskolin, 10 to 100 ng/M ℓ NGF, 1 \times supplementary element mixture and 1 \times 10 $^{-6}$ and 1 \times 10 $^{-5}$ % beta-mercaptoethanol.

11. (Original) The method of claim 9, wherein the multipotent progenitor/stem cells are inoculated into the animal cell culture medium at a concentration ranging from 2 \times 10 4 to 8 \times 10 4 cells/cm 2 and culturing at 37°C under an atmosphere of 5% CO $_2$ for 1 to 2 weeks.

12. (Original) An animal cell culture medium composition for differentiating multipotent progenitor/stem cells of claim 5 into neurons, which comprises 0.1 to 2% FBS, 1 to 2 mM L-glutamine, 1 to 25 μ M retinoic acid, 1 to 20 μ M forskolin, 10 to 100 ng/M ℓ NGF, 1 \times supplementary element mixture and 1 \times 10 $^{-6}$ to 1 \times 10 $^{-5}$ % beta-mercaptoethanol, in addition to D-glucose ranging from 3,500 to 5,500 mg/M ℓ and sodium pyruvate ranging from 50 to 200 mg/M ℓ .

13. (Original) A method for differentiating multipotent progenitor/stem cells of claim 5 into osteoblasts, which comprises culturing the multipotent progenitor/stem cells in an animal cell culture medium comprising FBS, dexamethason, ascorbate-2-phosphate and β -glycerophosphate, in addition to D-glucose ranging from 3,500 to 5,500 mg/M ℓ and sodium pyruvate ranging from 50 to 200 mg/M ℓ .

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14. (Original) The method of claim 13, wherein the animal cell culture medium contains 5 to 20% FBS, 0.1 to 1 μ M dexamethasone, 10 to 100 μ M ascorbate-2-phosphate and 5 to 20 mM β -glycerophosphate.

15. (Original) The method of claim 13, wherein the multipotent progenitor/stem cells are inoculated into the animal cell culture medium at a concentration ranging from 5×10^4 to 2×10^5 cells/cm² and culturing at 37°C under an atmosphere of 5% CO₂ for 2 to 3 weeks.

16. (Original) An animal cell culture medium composition for differentiating multipotent progenitor/stem cells of claim 5 into osteoblasts, which comprises 5 to 20% FBS, 0.1 to 1 μ M dexamethasone, 10 to 100 μ M ascorbate-2-phosphate and 5 to 20 mM β -glycerophosphate, in addition to D-glucose ranging from 3,500 to 5,500 mg/Ml and sodium pyruvate ranging from 50 to 200 mg/Ml.

17. (Original) A method for differentiating multipotent progenitor/stem cells of claim 5 into endothelial cells, which comprises culturing the multipotent progenitor/stem cells in an animal cell culture medium comprising FBS and vascular endothelial growth factor(VEGF), in addition to D-glucose ranging from 3,500 to 5,500 mg/Ml and sodium pyruvate ranging from 50 to 200 mg/Ml.

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18. (Original) The method of claim 17, wherein the animal cell culture medium contains 0.1 to 2% FBS and 10 to 100 ng/Mℓ VEGF.

19. (Original) The method of claim 17, wherein the multipotent progenitor/stem cells are inoculated into the animal cell culture medium at a concentration ranging from 1×10^5 to 4×10^5 cells/cm² and culturing at 37°C under an atmosphere of 5% CO₂ for 2 to 3 weeks.

20. (Original) An animal cell culture medium composition for differentiating multipotent progenitor/stem cells of claim 5 into endothelial cells, which comprises 0.1 to 2% FBS and 10 to 100 ng/Mℓ VEGF, in addition to D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

21. (Original) A method for differentiating multipotent progenitor/stem cells of claim 5 into myoblasts, which comprises culturing the multipotent progenitor/stem cells in an animal cell culture medium comprising bovine serum albumin(BSA) and 5-azacytidine, in addition to D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

22. (Original) The method of claim 21, wherein the animal culture medium contains 5 to 10% BSA and 10 to 20 μ M 5- azacytidine.

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23. (Original) The method of claim 21, wherein the multipotent progenitor/stem cells are inoculated into the animal cell culture medium at a concentration ranging from 1×10^5 to 5×10^5 cells/cm² and culturing at 37°C under an atmosphere of 5% CO₂ for 5 to 6 weeks.

24. (Original) An animal cell culture medium composition for differentiating multipotent progenitor/stem cells of claim 5 into myoblasts, which comprises 5 to 10% BSA and 10 to 20 μ M 5-azacytidine, in addition to D-glucose ranging from 3,500 to 5,500 mg/Ml and sodium pyruvate ranging from 50 to 200 mg/Ml.

25. (Original) A method for differentiating multipotent progenitor/stem cells of claim 5 into hepatocytes, which comprises culturing the multipotent progenitor/stem cells in an animal cell culture medium comprising hepatocyte growth factor(HGF), oncostatin M and L-glutamine, in addition to D-glucose ranging from 3,500 to 5,500 mg/Ml and sodium pyruvate ranging from 50 to 200 mg/Ml.

26. (Original) The method of claim 25, wherein the animal cell culture medium contains 10 to 100 ng/Ml HGF, 5 to 50 ng/Ml oncostatin M and 1 to 2 mM L-glutamine.

27. (Original) The method of claim 25, wherein the multipotent progenitor/stem cells are inoculated into the animal cell culture medium at a concentration ranging from 5×10^4 to 5×10^5 cells/cm² and culturing at 37°C under an atmosphere of 5% CO₂ for 2 to 4 weeks.

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28. (Original) An animal cell culture medium composition for differentiating multipotent progenitor/stem cells of claim 5 into hepatocytes, which comprises 10 to 100 ng/Mℓ HGF, 5 to 50 ng/Mℓ oncostatin M and 1 to 2 mM L-glutamine, in addition to D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

29. (Original) A method for differentiating multipotent progenitor/stem cells of claim 5 into dendritic cells, which comprises the steps of: culturing the multipotent progenitor/stem cells in a first animal cell culture medium comprising FBS, L-glutamine, GM-CSF and interleukin-4(IL-4) for inducing immature differentiation; transferring the immature differentiated cells in a second animal cell culture medium comprising FBS, L-glutamine, tumor necrosis factor- α (TNF- α), IL-1 β , IL-6 and prostaglandin E2; and culturing them for inducing mature differentiation, wherein each of the animal cell culture media further contains D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

30. (Original) The method of claim 29, wherein the first animal cell culture medium contains 1 to 2% FBS, 1 to 2 mM L-glutamine, 10 to 1,000 ng/Mℓ GM-CSF and 10 to 100 ng/Mℓ IL-4, and the second animal cell culture medium contains 1 to 2% FBS, 1 to 2 mM L-glutamine, 1 to 100 ng/Mℓ TNF- α , 1 to 2 ng/Mℓ IL-1 β , 100 to 1,000 U/Mℓ IL-6 and 0.1 to 10 μ g/Mℓ prostaglandin E2.

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31. (Original) The method of claim 29, wherein the multipotent progenitor/stem cells are inoculated into the first animal cell culture medium at a concentration ranging from 1×10^5 to 1×10^7 cells/cm² and culturing at 37°C under an atmosphere of 5% CO₂ for 3 to 15 days, and the immature differentiated cells are culture in the second animal cell culture medium at 37°C under an atmosphere of 5% CO₂ for 1 to 7 days.

32. (Original) An animal cell culture medium composition for differentiating multipotent progenitor/stem cells of claim 5 into dendritic cells, which is selected from the group consisting of:

an animal cell culture medium composition for inducing immature differentiation comprising 1 to 20% FBS, 1 to 2 mM L-glutamine, 10 to 1,000 ng/Mℓ GM-CSF and 10 to 100 ng/Mℓ IL-4; and

an animal cell culture medium composition for inducing mature differentiation comprising 1 to 20% FBS, 1 to 2 mM L-glutamine, 1 to 100 ng/Mℓ TNF- α , 1 to 100 ng/Mℓ IL-1 β , 100 to 10,000 U/Mℓ IL-6 and 0.1 to 10 μ g/Mℓ prostaglandin E2,

wherein each of the animal cell culture media further contains D-glucose ranging from 3,500 to 5,500 mg/Mℓ and sodium pyruvate ranging from 50 to 200 mg/Mℓ.

33. (Original) A cell composition for a cell therapy comprising the multipotent progenitor/stem cell of claim 5.

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34. (Original) The cell composition of claim 33, which is used for treating Parkinson's disease, Alzheimer's diseases, quadriplegia resulting from spinal cord injury, leukemia, apoplexy, encephalophyma, juvenile-onset diabetes, cardiac infarction, hepatocirrhosis, muscle diseases, cardiomyopathic diseases, liver diseases, blood diseases, the disruption and permanent functional disorder of osteoblasts and chondrocytes.